

Capsid Modified Bluetongue Virus 16 (BTV16) as a Virulytic Oncotherapy Agent

Taghi Naserpour Farivar,^{1,*} Reza Najafipour,¹ Safar Ali Alizadeh,¹ Seyyed Mahmoud Azimi,² and Pouran

Johari¹

¹Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, IR Iran

²Razi Vaccine and Serum Research Institute, Karaj, IR Iran

*Corresponding author: Taghi Naserpour Farivar, Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, IR Iran. Tel: +98-283324971, E-mail: taghin@yahoo.com

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Abstract

Objective: Using potential viruses to destroy cancer cells has a long history, but recent advances in molecular biology raised hopes for successful use of these viruses again.

Methods: Octreotate sequence was inserted into the neutralization region (R1& R2) in vp2 protein of capsid segment in 10 segmented genome of BTV in 304 - 368 position. T7 BTV RNA transcripts were extracted. Cancerous cultured cells were transfected with wild and modified BTV to recover BTV with cDNA-derived genome segments.

Results: The results of all the performed experiments revealed that treatment of AGS cell lines with VP2 modified BTV16, which targeted cell surface of cancerous cells, significantly increased apoptosis in cancer infected cells.

Conclusions: Modified VP2 BTV16 may be used as a potential virulytic oncotherapy agent in AGS cells.

Keywords: Bluetongue Virus, Oncotherapy, Virulytic, AGS

1. Background

Ability of viruses in delaying growth of human cancerous cells has a long history and dates back to early 19th century (1). In the late 19th century and for the first time, it was suggested that reovirus in in vitro conditions could selectively destroy selected human cancerous cells (2). This was a novel discovery and raised hope that viruses (3) with oncolytic potentials could be used as a killing machine to destroy human cancerous cells (4, 5). Previous studies suggested 6 major viral families: herpesviruses, flaviviruses, papillomaviruses, reovirus type 3, hepadnaviruses, and retroviruses with potential virulytic oncotherapy ability (6-10), all of which, except reovirus, are human cancer viruses.

There are not any reports on pathogenicity of bluetongue viruses to humans; moreover, it has been reported that bluetongue viruses are merely pathogenic to domestic cattle and wild ruminants (11). Thus, it is not surprising that there is not any pre-existing antibodies to BTVs in humans (12). Infection of human normal cells such as primary murine embryos fibroblast (MEF), the primary human embryo lung fibroblast (HEL), and Madin-Darby canine kidney (MDCK) with BTVs were not successful (11-13).

Octreotate is an octapeptide that pharmacologically looks like somatostatin (14, 15). Somatostatin and octreo-

tate are potentially active against several studied cancers including colorectal carcinoma due to their antiproliferative and apoptosis-inducing activity. When octreotate becomes radiolabeled, it can be used well for imaging of different types of cancers including breast, endocrine, neuroendocrine, small-cell lung, and prostate. It has also been used for targeted radiotherapy (16).

DeMaula et al. found that neutralization region (R1 & R2) in vp2 protein of capsid segment in 10 segmented genome of BTV is 304 - 368. Thus, we proposed to place octreotate in 321 - 346 position (17).

2. Objectives

The present study aimed at using BTV virus to genetically modify its spike through changing its capsid protein antigenic epitope to target cancerous cell lines and check apoptosis effect in infected host cells.

3. Methods

HeLa (human cervical epithelial carcinoma) cells were maintained in Dulbecco's modified Eagle's medium, containing 10% fetal calf serum (FCS), 100 U of penicillin/mL, and 100 µg of streptomycin /mL and cells were incubated at 37°C.

Neutralization region (R1 & R2) in vp2 protein of capsid segment in 10 segmented genome of BTv is 304 - 368 position. Thus, we proposed to place octreotate in 321-346 position in this segment. Therefore, in segment 2 gene of strain of bluetongue virus 9 (accession number: JN2559639), with a 2921 bp length, octreotate sequence was inserted in 1711 to 1734 bp position; and immediately before viral vp2 sequence a T7 promoter, and immediately after that a BsaI restriction site were inserted, and the whole sequence (2957 bp) was cloned into pUC57 (Cinna Gen, Tehran, Iran).

Competent Stella cells were transfected with pUC-30434-Naserpour Plasmid and incubated over night at 37 degree centigrade with 200 rpm shaking. Cells were harvested by centrifuging, and after lysis, plasmids were extracted with plasmid extraction kit according to its manual (Qiagen, Tehran, Iran). Extracted and purified plasmid was cut with BsaI restriction enzyme at 37/4h and electrophoresed in agarose gel 0.8%. The cut plasmids were extracted from gel by Qiagen gel extraction kit using its manual (Qiagen) and were used as template DNA to make RNA with mMESSAGE mMACHINE T7 Ultra kit (Ambion) using its manual (18) (Figure 2).

Then, T7 BTv transcripts were extracted and RNA pellets were washed twice in 70% (vol/vol) ethanol and dissolved in sterile DPEC treated water. Purified BTv single-stranded RNA (ssRNA) was analyzed by electrophoresis on 1% denaturing agarose using standard techniques (29) (Figure 3). Purified BTv single-stranded RNA was stored at -80°C.

Cultured cells were transfected to recover BTv with cDNA-derived genome segments. BTv mRNAs were mixed with T7 BTv transcripts in Opti-MEM I in the presence of 0.1 U/ μ L RNasin Plus (Cinna Gen, Tehran, Iran). The RNA mixture was incubated at 20°C for 30 minutes before being mixed with prepared liposome. Monolayers of HeLa cells in 6 well plates were transfected with 1.5 μ g of BTv mRNA, which was mixed with 0.75 μ g of T7 BTv transcript using prepared liposome reagent according to the manufacturer's instructions. Four hours after transfection, the culture medium was replaced with a 6-mL overlay consisting of minimal essential medium, 1.5% (wt/vol) agarose type VII (Sigma) and 2% FBS. Assays were incubated at 35°C in 5% CO₂ for 72 to 96 hours to allow plaques to appear.

To prepare dsRNA from transfection-derived BTv plaques, each plaque was picked into 500 μ L of Dulbecco's modified Eagle's medium containing 5% FBS, and 200 μ L was used to infect 1.5×10^6 AGS cells. Infected cells were incubated at 35°C in 5% CO₂ incubator for 72 to 96 hours to allow amplification of the BTv.

BTv serotype 16 (BTv-16), tittered by a plaque assay at 2.5×10^7 PFU/mL, was used for cell infection. First, cell monolayers were washed with FCS-free growth medium, and then cell monolayers were incubated with viruses at the

required multiplicity of infection (MOI). Virus adsorption was done for 2 hours, followed by incubation in growth medium supplemented with 5% FCS.

To detect chromosomal DNA fragmentation, approximately 2×10^6 AGS cells were resuspended and lysed in lysis solution, containing 0.5% Triton X-100, 5 mM Tris-HCl (pH 7.4), and 5 mM EDTA, and maintained for 20 minutes on ice. Nuclei were removed by centrifugation at $10,000 \times g$ for 15 minutes. Supernatants were treated with 50 μ g of RNase A/mL for 1 hour at 37°C, and DNAs were extracted by the standard phenol-chloroform extraction, and precipitated pellets were dissolved in Tris-EDTA (pH 7.5). DNAs with different size fragments were separated by electrophoresis in 2% agarose gels.

4. Results and Discussion

Plasmid map of pUC57, inserted with segment 2 gene of bluetongue virus 16, which was substituted with an octreotate sequence in position 1711 to 1734 bp, had a T7 promoter immediately before and BsaI restriction site immediately after vp2 gene (Figure 1).

This fragment had segment 2 gene of bluetongue virus 16 substituted with an octreotate sequence in position 1711 to 1734, a T7 promoter immediately before and BsaI restriction site immediately after VP2 gene.

Results of treating plasmid pUC-30434-Naserpour with BsaI or PaeI restriction enzyme are presented in Figure 2.

Figure 3 illustrates that T7 BTv transcripts with 2921 bp size were produced using mMESSAGE mMACHINE T7 Ultra Kit.

AGS cells were treated with normal and modified BTv16. Infection of AGS with modified BTv16, with an octreotate part in their VP2 protein, led to apoptosis in this cell (Figure 4).

Evaluation of apoptosis by Apoptotic DNA Ladder in AGS treated with Normal BTv16 and VP2 modified BTv16 revealed that DNA laddering and fragmentation were observed in AGS cells treated with VP2 modified BTv16 (Figure 5).

Virulytic oncotherapy has raised hope that viruses (Kim et al. 2006) with oncolytic potentials can be used as a killing machine to destroy human cancers cells (Liu, 2006; Holtz, 2007). In this study, VP2 modified BTv16 were prepared for this purpose, and the results of all the performed confirming experiments revealed that treatment of AGS cell lines with VP2 modified BTv16 which targeted cell surface of cancerous cells, significantly increased apoptosis in these cells. Thus, it is possible to use this virus as a potential virulytic oncotherapy agent.

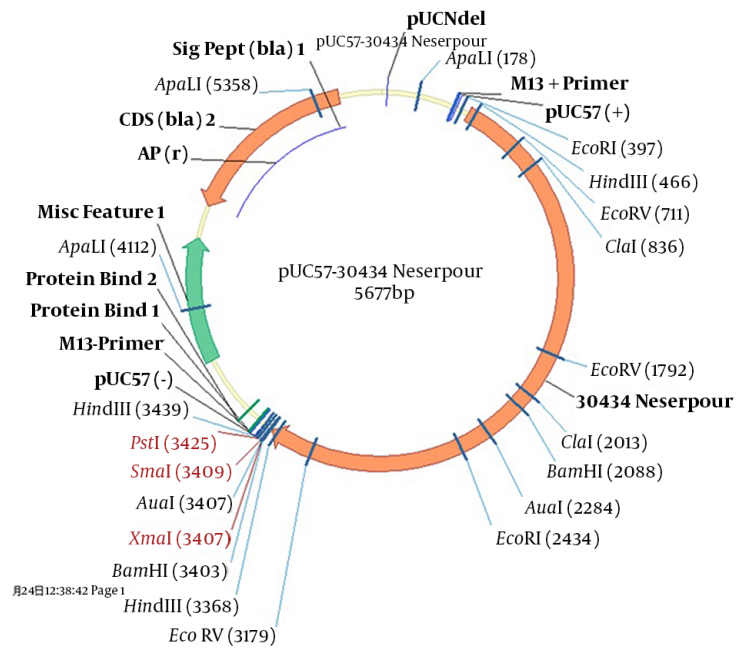


Figure 1. pUC57 with 30434-Naserpour Fragment

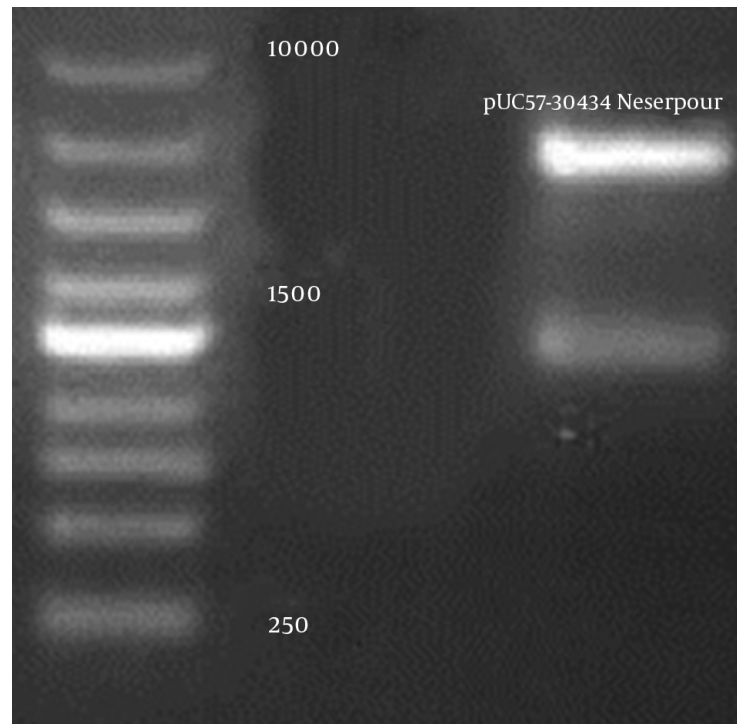


Figure 2. pUC57-30434 Naserpour Treated with BsaI or PstI Restriction Enzyme

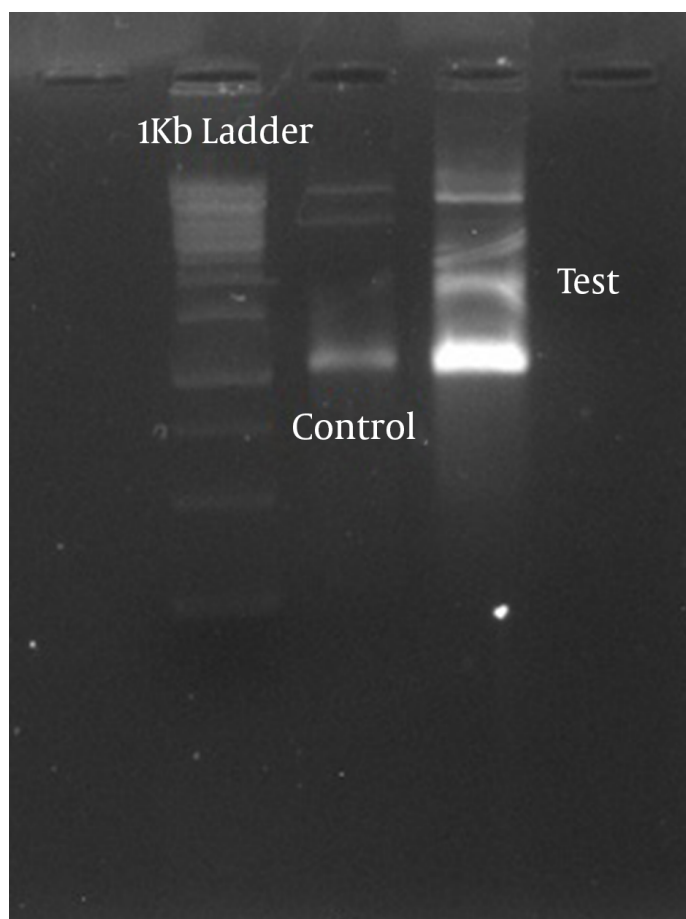


Figure 3. T7 BTV Transcripts of Octreotate Inserted VP2 of BTV16

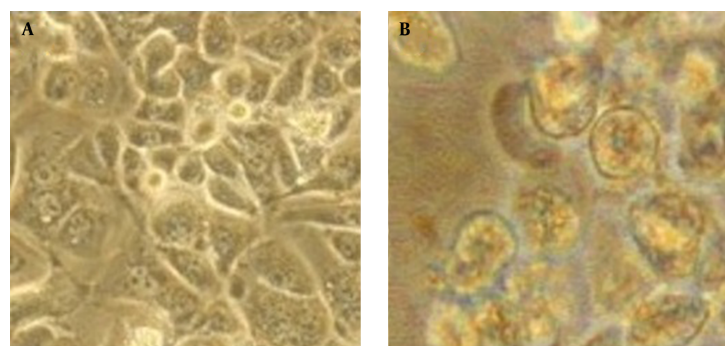


Figure 4. A: AGS Cells Treated with Normal BTV16. B: AGS Cells Treated with VP2 Modified BTV16

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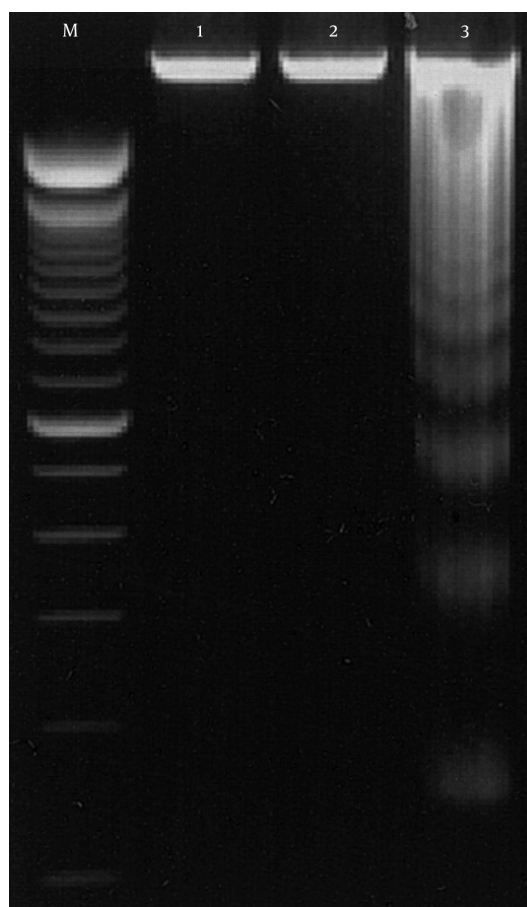


Figure 5. M, DNA Ladder; Line 1, Untreated AGS Cells; Line 2, AGS Treated With Normal BTV16; Line 3, AGS Treated With Spike Modified BTV16

ical Sciences.

Footnotes

Authors' Contribution: Taghi Naserpour Farivar designed the research, performed the experiment and analyzed the data; Reza Najafipour analyzed the data; Pouran Johari performed the experiment and analyzed the data; Seyyed Mahmoud Azimi prepared BTV16, and Safar Ali Alizadeh performed the experiment.

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